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EHC 7-31-95 #5

Primate models for HIV vaccines

Alan M. Schultz and Shiu-lok Hu*

AIDS 1993, 7 (suppl 1):S161–S170

Keywords: Vaccine, chimpanzee, macaque, simian immunodeficiency virus (SIV), HIV, animal model, AIDS.

Introduction

Experiments in animal models have clearly established that active (and passive) immunization can protect primates from intravenous challenge with cell-free immunodeficiency virus [1–7]. Protection from cell-associated virus challenge was demonstrated in chimpanzees and macaques in 1992. Recent results with live-attenuated vaccine strategies have also shown impressive protection against simian immunodeficiency virus (SIV) challenge [8]. Conceptually, in the SIV model at least, there is evidence that 'sterilizing immunity', or preventing the very first infectious event, may not be necessary for a vaccine to be effective. On the other hand, findings that there are protective immune responses to the cellular antigens present in whole-inactivated virus vaccine preparations have complicated the interpretation of the large body of successful protection experiments obtained with this vaccine approach in the SIV/macaque model [9–17].

Although progress was achieved in 1992, it is still difficult to predict from these studies how close we are to obtaining a successful human AIDS vaccine, for at least three reasons. Experiments in the chimpanzee model have been very limited, a correlate of protective immunity in the macaque model remains elusive, and the various models do not always give the same results in similar experiments. This review will summarize that progress, review discrepancies between similar experiments in the various models, attempt to clarify what can be learned from primate models for AIDS vaccines, and look forward to the focus of future work.

The animal models

A model consists of a virus and an animal species. For AIDS vaccine studies, the choice of primate species is limited to the chimpanzee and the several varieties of macaque. However, the choice of virus is equally important, and here the selection is considerably larger, leading to multiple inter-related models. HIV-1/chimp is the first choice for an animal model, since the human epidemic is primarily an HIV-1 epidemic, and HIV-1 grows in chimpanzees [18–20]. However, there are several limitations to the use of chimpanzees: (1) the substantial expense severely curtails the amount of experimentation; (2) chimpanzees show no persistent sign of HIV-related disease, and HIV isolation becomes increasingly difficult with time in many animals; (3) currently only one HIV-1 isolate (HIV_{LAI}) has been used to challenge chimps, and most experiments have used a single challenge stock of that isolate [21]. Efforts are being made to increase the number of HIV-1 challenge stocks, and this variety will improve the power of the model.

In macaques, the bulk of the immunization effort has been invested in various SIV isolates, although some work has been performed with independent isolates of HIV-2 [22–26]. Virologically and genetically, HIV-2 is much more similar to SIV than to HIV-1 [27,28], and reproducible disease has not been associated with HIV-2 infection of macaques. Thus, despite being a human pathogen, HIV-2 is of significantly less intrinsic interest than HIV-1 for vaccine studies. Nonetheless, some significant experimental results have been obtained and will be reviewed below.

Two discoveries made in 1992 have raised hopes that direct evaluation of candidate HIV-1 vaccines may be possible in macaques. *Macaca nemestrina*, a macaque subspecies that is bred in captivity and can still be obtained from the wild in Indonesia, has been shown to support replication of HIV-1 [29]. Attempts to duplicate the *in vivo* results have met with mixed success. At a recent workshop (the Fifth Annual National Co-operative Vaccine Development Groups Meeting at Chantilly in August/September 1992) it appeared that large amounts of virus may be necessary to initiate a successful infection with HIV-1, and that virus replication wanes after several months [30]. It is likely that in the next year, work will concentrate on improving the reproducibility of this new model in other labo-

From the Vaccine Research and Development Branch, Division of AIDS, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland and *Bristol-Myers-Squibb Pharmaceutical Research Institute, Seattle, Washington, USA.

Requests for reprints to: Alan M. Schultz, Vaccine Research and Development Branch, Division of AIDS, NIAID, NIH, Room 2B-01, 6003 Executive Boulevard, MD 20892, USA.

ISBN 1-870485-74-2 ISSN 0269-9370

ratories and on looking for any long-term effects of infection. The second discovery was the successful insertion of the HIV-1 envelope gene into an SIV genetic background to produce a chimeric virus that can replicate in macaques [31–33]. This chimeric virus requires a lower input dose to initiate infection, but the duration of the viremia after infection requires further investigation. An attractive feature of this model is the expectation that many variant HIV-1 envelope genomes could be substituted into the same genetic background, constituting a powerful tool for evaluation of the breadth of protection in vaccinated animals. Further refinements in both these new models are awaited with great anticipation.

Virus isolates

For AIDS vaccine development, most experimentation has been done with SIV infection of the macaque because it duplicates the human disease syndrome, and large numbers of animals can be studied. To date, hundreds of macaques have been immunized with various vaccines and challenged with SIV variants [1]. The variants differ by approximately 15% in envelope protein sequence [28,34–36], which allows for testing protection against disparate challenge viruses. However, these variants also differ in biological and immunological properties, which may introduce significant but unknown variables into the models.

SIV isolates that cause disease in macaques occur in one of three groups, depending on their source: SIV_{sm}, SIV_{mac}, and SIV_{mne}. The SIV_{sm} isolates (Fig. 1) come directly from sooty mangabeys (*Cercocebus atys*), which in captivity often harbor an immunodeficiency virus that has no deleterious effect; passage into a macaque causes immunodeficiency, wasting, and eventually death [37,38]. A virus named B670, derived after rhesus-to-rhesus passage of a mangabey virus, has been used extensively at the Delta Primate Center, Covington, Louisiana, USA [39,40]. Another laboratory has used E660, an SIV_{sm} derived from a second mangabey (E038) at Delta [39,41]. Figure 1 also shows sm H4, an infectious molecular clone [42]. Other isolates not shown in Figure 1 were similarly derived in the course of these experimental passages, but B670 and E660 were chosen for further use on the basis of their ability to reproducibly induce disease with a rapid onset (usually 6–18 months to death). Among viruses obtained from mangabeys at the Yerkes Primate Center, Atlanta, Georgia, USA, the rapidly pathogenic PBj14 virus [43,44] has not yet been used in vaccine studies. SMM-3 and SMM-7, independent isolates from two mangabeys [45], have been used in vaccine studies in Europe.

The SIV_{mac} family is more complex (Fig. 2), although the isolates used in vaccine work originated from a sin-

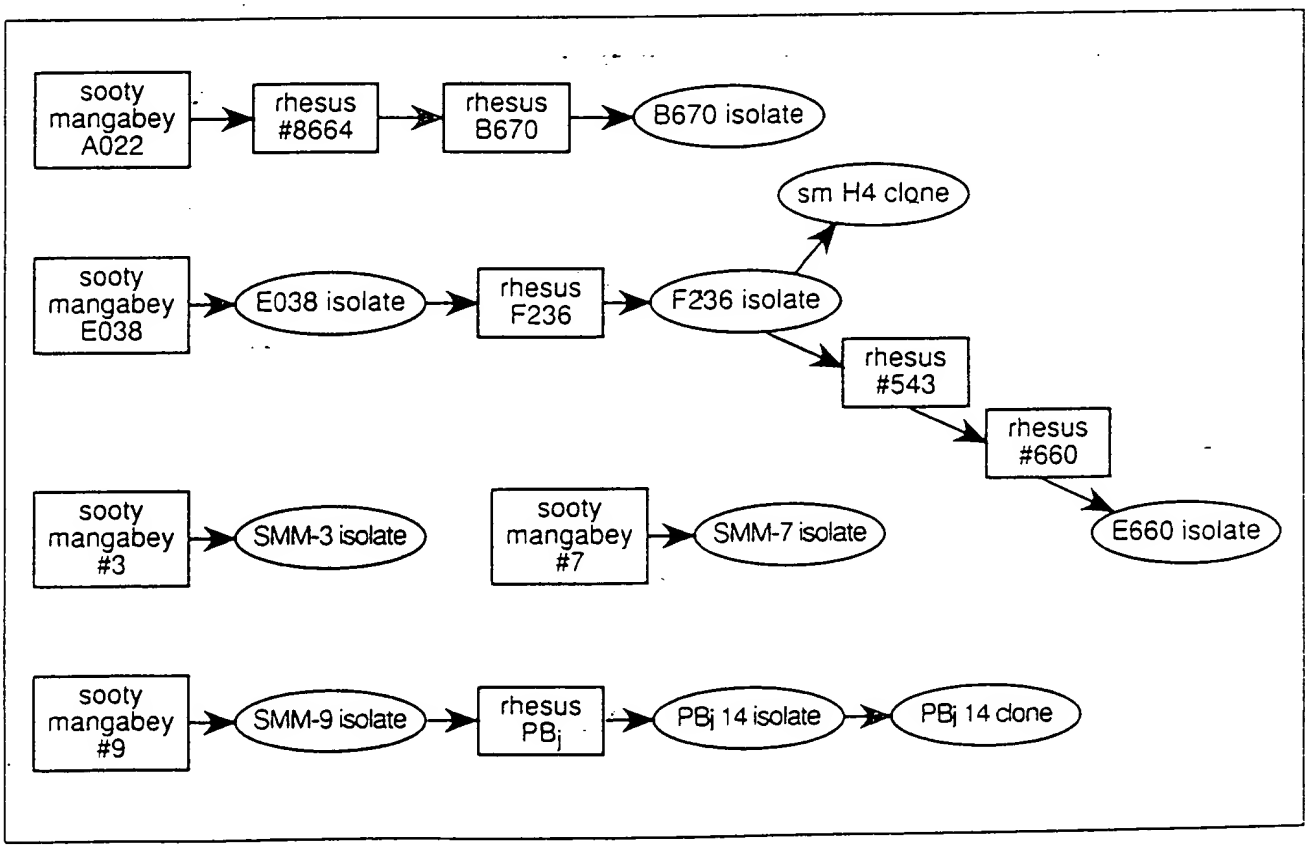

Fig. 1. Derivation of SIV_{sm} isolates.

gle rhesus macaque at the New England Primate Center, Southborough, Massachusetts, USA, where the SIV disease syndrome was first described [46,47]. Rhesus-to-rhesus passage resulted in isolate SIV_{mac251} [48], which is the most commonly used SIV_{mac} isolate. Isolate 32H, derived by an additional animal passage of isolate SIV_{mac251}, has been used extensively in the United Kingdom [49]. The precise derivation of molecular clones BK28 [50] and 1A11 [51] is difficult to trace, but the nucleotide sequence of their envelope genes leaves little doubt that they are clones of SIV_{mac251}. Serial animal passage of SIV_{mac251} produced SIV_{mac239} [47], which has been the source of several important virus isolates. SIV_{mac239} itself, and SIV_{mac239} 'open', are pathogenic in macaques; SIV_{mac239} Δ *nef* is not [52,53]. SIV_{mac239} also provides the SIV background used to create the chimeric HIV/SIV viruses [31,32], designated 'SHIV' in Fig. 2. The 316 isolate of SIV_{mac} is a macrophage-tropic virus [54] that has not yet been used in vaccine studies. Like SIV_{sm}, more virus isolates have been recovered than are shown in Fig. 2; those chosen for further use as vaccine challenges induce a rapid-onset, fatal (typically within 6–18 months) disease.

SIV_{mne}, isolated from a pig-tailed macaque that died of lymphoma [55], differs from other SIV in two ways. SIV_{mne} did not arise after intentional animal-to-animal intravenous passage of infectious material, and it induces a somewhat slower disease progression than the other SIV. SIV_{mne} induces wasting, CD4 cell depletion, and is ultimately fatal, but infected animals usually survive at least 2 years before becoming moribund [56]. One biological clone, E11s, has also induced fatal disease [57]. By sequence, its envelope is somewhat closer to SIV_{mac} than to SIV_{sm} [34].

A fourth family of pathogenic viruses, SIV_{stm}, has recently been described in molecular terms. This virus was isolated from stump-tailed macaques (*M. arctoides*), in which a horizontal, fatal epidemic of immunodeficiency and lymphoma occurred [58]. In molecular terms, it is equidistant from SIV_{sm} and SIV_{mac}/SIV_{mne} [59,60] and may be suitable for cross-protection SIV test challenge stocks in the future.

Two further groups of SIV isolated from monkeys deserve brief mention, although they do not appear to be pathogenic in the host species or in macaques. The African green monkey has yielded a wide variety of SIV isolates [61–66], and an SIV has been isolated from the Sykes monkey (*Cercopithecus mitis*) [67].

As discussed briefly above, HIV_{LAI} is the only titered stock of HIV-1 currently available for chimpanzee challenge. HIV_{MN} has proven refractory to growth in chimpanzees [68], but an HIV_{SF2} challenge stock titration is nearing completion (K.K. Murthy, personal communication, 1993). HIV_{LAI} and HIV_{MN} have been passed

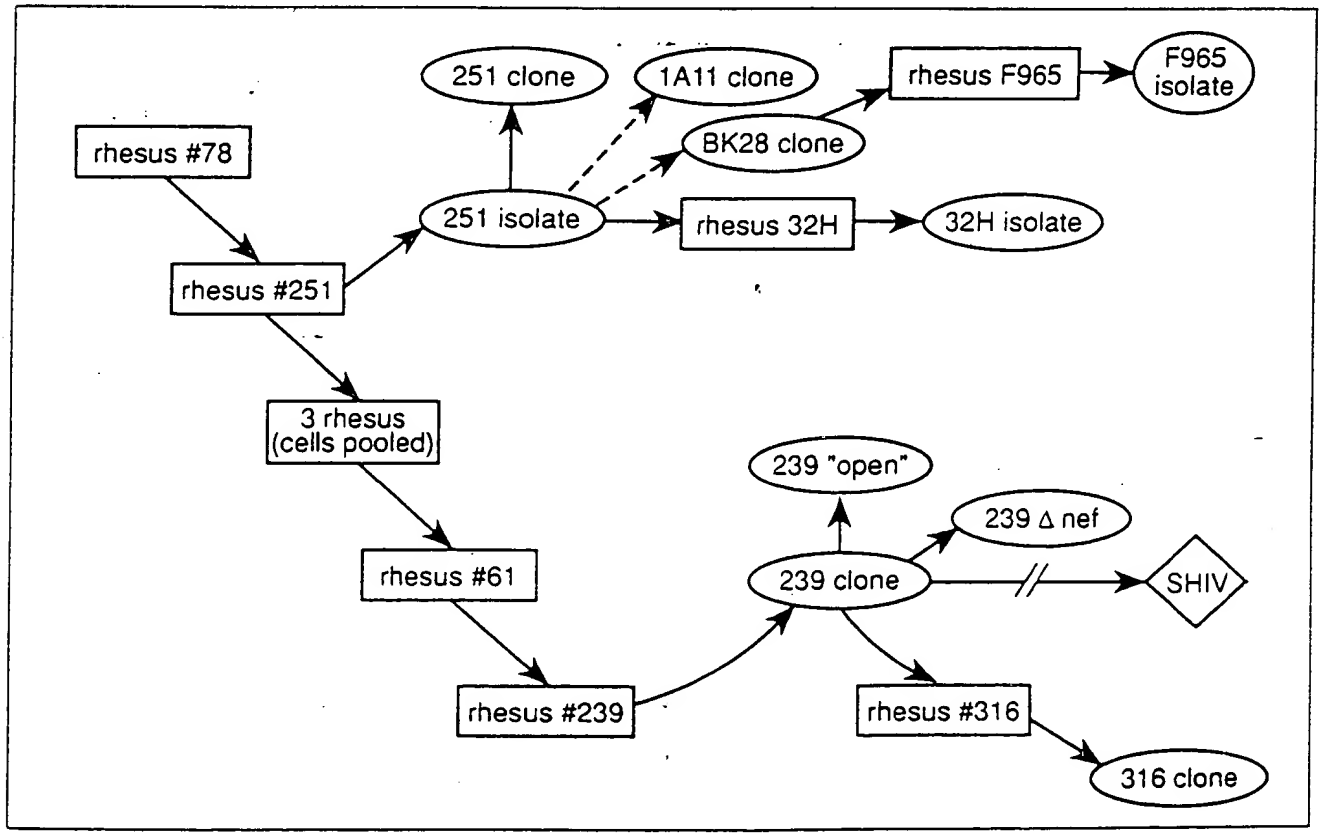

Fig. 2. Derivation of SIV_{mac} isolates.

be more responsive than C339. Until vaccines can induce protection against higher doses of challenge virus, it will be difficult to draw definitive conclusions from experiments with such a small number of animals.

A very recent experiment similar in design to the earlier Genentech experiment [79] compared gp120 and gp160 vaccines (C. Bruck, personal communication, 1992). In this experiment, two chimpanzees immunized with insect-cell-derived gp120 were infected after challenge with HIV_{LAI}, but one of two animals immunized with mammalian-cell-derived gp160 was protected from infection. During the course of immunization the four chimps responded with different neutralization titers for HIV_{LAI}. The protected chimpanzee had a considerably higher neutralization titer than the others.

Results of experiments with recombinant subunit vaccines remain disappointing in the SIV/macaque model. In several independent experiments, macaques immunized with gp120 or gp160 subunit vaccines and subsequently challenged with SIV_{mac} or SIV_{sm} have not been protected [82,83] (E. Hoover, M. Murphey-Corb, personal communications, 1992). No similar experiment has been attempted in an SIV_{mne} system.

Live recombinant vaccinia

Since the failure of the early experiments, which used a rather high HIV challenge dose, this approach has not been tested in chimpanzees [84]. A chimp immunized with vaccinia expressing the HIV *env* gene and then boosted with recombinant subunits and synthetic V3 peptides appeared to be protected, but eventually became positive for HIV isolation 8 months after challenge [80]. The only successful protection in primates with vaccinia has been obtained by boosting vaccinia *env*-immunized macaques with gp160 partially purified from a baculovirus vector and challenging with a low-dose of SIV_{mne} clone E11s [85,86]. One year later, in 1992, these four successfully protected animals were re-boosted with gp160 and challenged with a 10-fold higher dose (100 animal infectious doses) of the parent SIV_{mne}, which contains a mixture of related viruses. Two of the macaques clearly became infected (positive for virus isolation and by PCR), one was apparently completely protected (negative for virus isolation and by PCR), and the fourth was positive for virus isolation at 2 weeks post-challenge but not subsequently.

In unpublished work, three other groups have tested the concept of priming with vaccinia and boosting with subunit proteins, followed by challenge with SIV_{mac} (E. Hoover, R. Desrosiers, T. Yilma, personal communications, 1992). No successful protection has been observed in these experiments, although indication of lower virus load after challenge in the vaccinated animals than in the naive controls was found by two groups.

Whole-inactivated virus

A major objection was raised in 1992 to the interpretation of the large body of experiments showing protection based on vaccines consisting of whole-inactivated SIV vaccines or SIV-infected cells [87–91]. All retroviruses, including SIV and HIV, are known to bud from cell membranes and to incorporate cell surface antigens in their lipid envelopes [92–94]. For experimental purposes, SIV has primarily been grown in human T-cell lines or human PBL, because most macaques have a relatively small blood volume and are not a convenient source of cells for large-scale growth of SIV. Thus, whole-cell or whole virion vaccines from human cells have contained human cellular antigens, to which macaques have responded after vaccination. The evidence that the observed protection was due to immune recognition of the human antigens found in the vaccine and common to the SIV challenge virus grown in human cells is fourfold: (1) two out of four macaques immunized with uninfected human c8166 cells containing no SIV antigens were protected when challenged with SIV_{mac} grown in c8166 cells [87] (E.J. Stott, personal communication, 1992); (2) four out of four macaques immunized with HIV-1 grown in c8166 cells were protected when challenged with SIV from c8166 cells [95]; (3) every macaque previously protected from SIV grown in human cells became infected when challenged with SIV grown in rhesus macaque cells, despite being re-boosted with inactivated vaccine from human cells before challenge [88,89] (P.R. Johnson, personal communication, 1992); (4) passive transfer of antibody from SIV-infected macaques did not protect naive macaques from SIV_{mac} grown in human cells, but antibody from macaques immunized with inactivated human-grown virus vaccine did (M.B. Gardner and P.R. Johnson, personal communications, 1992).

Two avenues of research are now being followed to resolve the issue. The first is aimed at identifying which cellular antigens are responsible, and it is possible that a novel vaccine approach may emerge from these studies. The second is to eliminate foreign antigens from the experiment. To test the role of SIV antigens alone requires production of the vaccine in monkey cells and to challenge with monkey-grown SIV; the herculean task of vaccine production is underway (M. Murphey-Corb, personal communication, 1993).

Despite this development, whole-inactivated vaccines have nonetheless shown protection in two other types of experiments not confounded by the cellular antigen problem. The first reported success of challenging vaccinated animals with cell-associated virus was with macaques immunized with whole-inactivated SIV vaccines [96,97]. In this experiment, eight macaques were immunized with inactivated SIV_{mac} in one of two adjuvants. All four controls became infected after challenge with cells from a moribund SIV_{mac}-infected macaque, but two out of four animals in each arm of

the vaccine experiment were protected. This result has been independently confirmed by a similar experiment with a whole-inactivated SIV vaccine, in which monkey PBL infected with SIV_{sm} were used as challenge. Control animals became infected, but seven out of nine vaccinees were protected (M. Murphey-Corb, personal communication, 1992). Although the vaccinated macaques showed anti-human immune responses in these experiments, this should not affect the challenge with SIV-infected rhesus macaque cells. The observed protection is clearly dependent on the low challenge dose, since no protection was observed in another experiment also using an inactivated SIV vaccine but a fivefold higher challenge dose of SIV-infected macaque cells [98].

In the HIV-2/macaque model, several trials with whole-inactivated vaccines have shown partial protection against challenge with monkey-grown free virus [99,100] (G. Biberfeld, personal communication, 1992). Previously, these experiments were not considered as important as the SIV trials with inactivated vaccines, which protected 100% of the vaccinees. However, the HIV-2 experiments are not compromised by non-specific responses to human antigens. To achieve this incomplete level of protection, more than the four doses of vaccine typically used in the SIV experiments were necessary. Additional work is required to establish how effective a vaccine of this type will be.

Live-attenuated vaccine

This approach has been highly successful for other viruses, although concern about the safety of an attenuated immunodeficiency virus has minimized serious attention to developing this type of vaccine for HIV. However, if a live-attenuated vaccine produces robust protection in animal models, it could provide valuable information about what immune responses are necessary for protection and could eventually lead to an HIV vaccine. Recent experiments in the SIV model with live-attenuated vaccines have demonstrated impressive protection against SIV challenge. Most molecular clones of SIV are avirulent in macaques, and two SIV_{mac} clones, 1A11 and SIV_{mac239} Δ *nef*, have been investigated as live vaccines. The exact cause of 1A11 avirulence is not known [101], but the *nef* deletion of SIV_{mac239} Δ *nef* clone is responsible for its failure to cause disease [53]. SIV_{mac239} Δ *nef* infection results in a serological response resembling that seen after active infection, and macaques remain PCR-positive, though virus isolation is rare from vaccinated animals. 1A11 infection is less persistent, and animals exhibit lower antibody levels and often become PCR-negative after many months.

The 1A11 attenuated vaccine had previously been shown capable of protecting macaques against extremely low-dose [102], but not high-dose [103], intravenous challenge with SIV_{mac}. In 1992, macaques immunized with either 1A11 or SIV_{mac239} Δ *nef* at least 18 months previously were challenged with the same stock of SIV_{mac251} grown in monkey cells (10 animal infectious doses) that had infected all previously apparently protected monkeys vaccinated with whole-inactivated vaccines grown in human cells (see above). After challenge, all 1A11-immunized animals became positive for SIV isolation for several weeks, but since then several have become negative for SIV isolation; however, they remain PCR-positive 6 months post-challenge (M. Marthas, personal communication, 1992). In the other experiment, two of the SIV_{mac239} Δ *nef*-immunized animals were challenged and two with the same SIV_{mac251} stock with SIV_{mac239} grown in the same monkey cells. Three of the four animals showed total protection with no sign of infection, and one yielded a small amount of virus 2 weeks after challenge but not subsequently. These four animals have been re-challenged with 1000 infectious doses and remain solidly protected 4 months post-challenge [8].

$HIV\text{-}2_{SBL/K135}$ produces a transient infection of cynomolgus macaques [100], and may be considered an attenuated vaccine. When cynomolgus macaques exposed to $HIV\text{-}2_{SBL/K135}$ were challenged with SIV_{sm}, they became infected but showed no disease progression [99,104]. This is an intriguing result, because HIV-2 and SIV_{sm} are not highly related to one another by molecular sequence comparison. In an update of this experiment 3 years after challenge, all four control animals challenged with SIV_{sm} have died, and all three vaccinated animals are healthy — two remain negative for virus isolation (G. Biberfeld, personal communication, 1992).

Discussion

The objective of a vaccine is to reduce morbidity and break the chain of transmission. It has been assumed that for a virus like HIV, which can establish persistent infection, a successful vaccine must achieve 'sterilizing immunity', whereby sufficient immunity is maintained to prevent the very first infections. Sterilizing immunity has been demonstrated experimentally in both HIV-1/chimpanzee and SIV/macaque models. However, it is not clear whether such immunity is necessary, or, more importantly, even attainable in practice. Evidence from the SIV/macaque model indicating that the goal of vaccination can be achieved without 'sterilizing immunity' has accumulated over the past year. Immunized animals that have become infected after challenge not only showed reduced virus load compared with control animals, but have also remained disease-free for prolonged periods. In some cases, attempts to re-isolate virus or to transmit infection experimentally from such animals have also failed. We propose that a successful vaccine, especially a first-generation one, may establish immunity in vaccinees who, upon exposure, may develop only transient in-

fection, or may become PCR-positive while remaining negative for virus isolation. Thus, they could remain disease-free and unlikely to transmit HIV to others. It remains an important task to understand the immune mechanisms that correlate with protection and to study the basis for long-term survival and possible viral clearance in HIV-infected humans and in SIV-infected macaques. In this regard, study of live-attenuated SIV vaccines and how they work may hold the key to future vaccine developments.

Subunit vaccines appear to have been effective in chimpanzees but not in macaques. However, it should be pointed out that the protective value of these vaccines in the chimpanzee model has been tested only under very limited conditions and with a challenge virus of minimal heterogeneity. Protection against heterologous virus has not been demonstrated and will have to wait for additional challenge stocks, which should be available shortly. Even when subunit vaccines appear to have been effective, the duration of protection against cell-free virus challenge is disappointing, since the single chimpanzee (C339 [81]) that was protected from cell-associated virus challenge became infected when rechallenged 1 year later with cell-free HIV without prior re-vaccination. Protection against mucosal infection has not been demonstrated, but relevant experiments are planned. We anticipate that a clearer picture will emerge in the chimpanzee model in the coming year, when heterologous challenges and at least one additional experiment studying the duration of protection will be complete.

Conflicting data were also obtained with protection against cell-associated virus challenge. Three groups [98] (E.J. Stott, M.B. Gardner, personal communications, 1992) reported that macaques immunized with whole-inactivated SIV and previously shown to be protected against cell-free virus challenge (from human cells) were readily infected when subsequently challenged with cells (PBMC or spleen cells) from SIV-infected animals. However, other data in both chimps and macaques suggest that this is not always the case. Two other groups observed partial protection in macaques; a total of 11 out of 17 are protected against infection with SIV-infected monkey cells [97] (M. Murphey-Corb, personal communication, 1992). Chimpanzee C499 [81] was protected from infected cell challenge 1 year after the most recent vaccination, whereas chimpanzee C339, as recounted above, was not protected when similarly challenged with free virus. This implies that immune responses that inactivate infected cells can be protective, and that neutralizing antibodies may not be the most essential component of protective immunity. Thus, protection against cell-free virus challenge may be harder to achieve than protection from cell-associated challenge. This observation may auger well for the eventual success of a vaccine against natural transmission, if the primary mode is through infected cells rather than free virus. However, the very low virus dose expected in human sexual exposure is difficult to model effectively in animal systems.

Another discrepancy in the SIV model concerns the protective efficacy of recombinant subunit vaccines, especially the approach using vaccinia for priming and subunit protein for boosting. While the SIV_{mne} model has given positive results [86], failure to obtain protection was observed by several groups in the SIV_{mac} model. Although half of the animals were infected when re-boosted and challenged with uncloned SIV_{mne} in the experiment recounted above, it should be noted that a large challenge (100 animal infectious doses) of SIV_{mne} was used. Previously, other SIV vaccines that were unsuccessful against such high-challenge doses showed protection when lower doses were used. This reduction in experimental challenge dose appears to be justified because the dose in human sexual exposure is presumed to be very low. Yet the trials in the SIV_{mac} model using low-challenge doses, even with minimal heterogeneity in the challenge (i.e., cloned virus), failed to prevent infection. This discrepancy between the SIV_{mac} and SIV_{mne} experiments could be due to subtle differences in vaccine or trial design. However, it is more probably due to differences in the biological properties of the two isolates. Although both SIV_{mne} and SIV_{mac} are pathogenic in multiple macaque subspecies, the kinetics of disease development appear to be different. It has been noted that SIV_{mne} takes longer to cause disease than SIV_{mac}. If this property is reflected in very early events in infection, SIV_{mne}-infected animals may have a better opportunity than SIV_{mac}-infected animals to establish an effective immune response to prevent or clear infection.

This hypothesis is supported by the results obtained in macaques immunized with peptide vaccines consisting of conserved envelope sequences. Disparate results were obtained depending on whether SIV_{mac} or SIV_{mne} was used as challenge. When challenged with between one and 10 animal doses of SIV_{mne} clone E11s, two out of three macaques experienced a transient infection and one was totally protected from infection, whereas all macaques became infected in another experiment when challenged with SIV_{mac251} at the same dose. These results indicate that pre-existing immunity should be more effective against a less aggressive virus, such as SIV_{mne}, which has not been selected for rapid progression by animal-to-animal passage. The SIV_{mac}-infected animals also exhibit reduced virus load and potentially slower disease progression than non-immunized controls, again indicating the existence of protective immune responses that are at least partially effective against infected cells. At this stage, it is not clear whether SIV_{mne} or SIV_{mac} would more closely represent HIV-1 infection in humans. Perhaps emphasis should be placed on studies with both viruses to gain insight into the complexity and diversity of primate lentivirus infection.

Conclusions

The development of primate models for AIDS vaccines progressed incrementally in 1992, though not as fast as one would hope, given the worldwide progression of the epidemic. Results from the various models do not give the same indications about which vaccines are most likely to work in humans, reflecting our increased appreciation of the complexity and diversity of primate lentivirus infections. Protection against cell-associated virus has been shown in the HIV-1/chimp model. However, the duration of protection, and protection from heterologous and mucosal challenges, remain to be demonstrated. In the past year we also saw the beginnings of macaque models for HIV-1 (the SHIV model and HIV infection of pig-tailed macaques). The usefulness of these models still needs to be explored. In the SIV model, the search for correlates of vaccine protection continues. The role of anti-cell antibodies, now confirmed by several laboratories, is still elusive. On the other hand, protection with vaccines has been clearly demonstrated using several approaches, including live-attenuated vaccines. Even though the eventual likelihood of attenuated vaccines for human use is debated, they remain powerful tools for unravelling protective immunity and providing a standard for other vaccine approaches to attain. Finally, results from the SIV model indicate that vaccinated animals, even if infected upon exposure, show reduced viral load, prolonged disease-free periods, and failure to transmit to naive animals. This suggests that the goals of vaccination (to reduce morbidity and transmission) could be attained without achieving 'sterilizing immunity'. Whether this will be true for any candidate HIV-1 vaccine in 'field' conditions needs to be considered as efforts are being gathered for efficacy trials in humans. Early in 1993 we maintain a cautious optimism that the likelihood of an AIDS vaccine is not a question of whether, but when.

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